

This document was developed by NIEHS/NTP staff to facilitate internal and external review of a proposed research program prior to designing and conducting toxicology studies. The purpose of the research concept document is to outline the general elements of a research program that would address the specific public health concerns that prompted the nomination of the substance or issue for study. It may also encompass substance-specific studies that address larger public health issues or topics in toxicology. Additional information about the nomination, review, and selection of substances for study by the NTP is provided at *Nominations to the NTP Testing Program* (<http://ntp.niehs.nih.gov/go/nom>). A draft version of this research concept was reviewed by the NTP Board of Scientific Counselors at a public meeting on June 22, 2007 (<http://ntp.niehs.nih.gov/go/9741>), subsequently revised, and approved by the NTP Executive Committee.

---

## **NTP Research Concept: Naturally Occurring Asbestos and Related Mineral Fibers**

### **Project Leader**

Scott Masten, Ph.D., DABT

DIR/ETP/OPD/Office of Chemical Nomination and Selection

### **Nomination Rationale and Background**

Naturally occurring and atypical forms of asbestos were nominated in 2006 by the National Center for Environmental Health/Agency for Toxic Substances and Disease Registry and the U.S. Environmental Protection Agency. These nominations were based on widespread community exposure to Naturally Occurring Asbestos (NOA) in certain geographic locales and insufficient dose-response information to characterize risk from exposure to non-commercial and “unregulated” asbestiform mineral fibers. The preliminary study recommendations are for mineral characterization, *in vitro* durability and toxicity studies, and subchronic and chronic toxicity/carcinogenicity studies via inhalation (<http://ntp.niehs.nih.gov/go/29287>). Both agencies expressly requested studies that will inform whether there are toxicological differences in mineral particles that are considered asbestiform and those that are not asbestiform but are of similar chemical composition (mineralogy) and dimension. The U.S. EPA nomination mentions several specific minerals of interest, including Libby amphibole, edenite, ferroactinolite, anthophyllite, tremolite, winchite, richterite, and erionite (U.S. EPA, 2006).

Asbestos is a generic commercial term describing several silicate minerals where crystalline growth produces very long, thin, flexible, separable fibers. The six asbestos minerals recognized by regulatory authorities are the fibrous serpentine mineral, chrysotile, the amphibole minerals crocidolite (riebeckite asbestos) and amosite (cummingtonite-grunerite asbestos), and the asbestiform varieties of the amphibole minerals tremolite, actinolite, and anthophyllite. Non-asbestiform varieties of amphibole minerals consist of crystalline particles of various morphology (e.g. prismatic, acicular) and dimension, some of which may be “fiber-like”. The term cleavage fragment is used for elongated particles that break off from massive forms of non-asbestiform amphibole minerals along specific crystallographic planes, usually as the result of mechanical processes, e.g. during commercial processing. Asbestiform minerals occur naturally in many parts of the United States (ATSDR, 2007), and community exposure to asbestiform fibers can increase in areas where the earth is disturbed through mining, building, recreation or other activities. These minerals rarely occur in nature as homogenous mineral deposits and exist as a continuum of particles of varying sizes, shapes, crystalline structure, and chemical composition. Two geographical areas of current public health scrutiny, Libby, MT and El Dorado County, CA, highlight some of the issues faced and the gaps in knowledge. Libby is the site of a former W.R. Grace vermiculite mine. From the early 1920s until 1990, more than 5

million tons of vermiculite ore was shipped to >200 processing facilities throughout the U.S. The Libby vermiculite ore contained substantial amounts of asbestiform and non-asbestiform fibrous amphiboles (winchite, richterite), and minor amounts of asbestiform tremolite, prismatic and acicular tremolite crystals and amphibole cleavage fragments. The incidence of asbestosis, lung cancer, and mesothelioma in this community is higher than would be expected (ATSDR, 2003a). However, the relative contribution of these different mineral particle exposures to disease cannot be ascertained from the human studies. In El Dorado, NOA exposures have been documented in air and soil at a local school and community recreation areas (U.S. EPA, 2005); however, there have been no studies to evaluate asbestos-related disease in this community and the health implications of these exposures continue to be debated. The USGS has characterized the amphibole particles in El Dorado as actinolite, magnesio-hornblende and tremolite, occurring in asbestiform and non-asbestiform fibrous morphologies (USGS, 2006). The USGS study also demonstrates that mineral particles labeled cleavage fragments by others are in fact prismatic and acicular single crystals rather than mechanically broken fragments of massive material.

Asbestos exposure is associated with a spectrum of pulmonary and thoracic disease including lung cancer, mesothelioma, asbestosis, and pleural disease. Evidence for an association between asbestos exposure and certain other cancers is suggestive but considered insufficient (Institute of Medicine, 2006). Exposure to other fibrous minerals such as erionite and some synthetic vitreous fibers has also been associated with adverse pulmonary effects including lung cancer.

Toxicology studies of “non-regulated” amphibole minerals such as winchite and richterite are lacking although these minerals are known to occur in the asbestiform habit. Of the available epidemiological and animal toxicological studies on asbestos, many included exposure to both asbestiform and non-asbestiform particles including, in some cases, cleavage fragments. Because of mixed and incomplete characterization of exposures in many of these studies, it has been difficult to relate specific adverse effects to specific sizes and types of fibers. Thus, public health and regulatory agencies have been unable to make clear distinctions regarding the relative hazard/risk of mineral particles of differing mineralogy, morphology, and dimension. A public health conservative approach would consider the hazard of similarly shaped and mineralogically identical particles as equivalent to the regulated asbestos minerals; however, agencies have been pressured to discount cleavage fragments and other non-asbestiform particles.

### **Rationale for Study**

Despite the known hazards of asbestiform fibers, exposures continue in certain occupations and environmental settings. The primary concern relative to this nomination is the community exposures to past and current residents of Libby, MT, former workers at the W.R. Grace vermiculite mine in Libby and vermiculite processing facilities around the country, and residents of areas where exposures to natural mineral fibers have been documented, e.g. El Dorado Hills, CA. The primary media in which asbestos minerals are found in these areas is soil, outdoor air, indoor dust, and indoor air. There is intense public concern regarding potential health risks from exposure to asbestos as a result of routine and recreational activities in these communities. Exposures to children are of special concern. Other stakeholders, e.g. building and building products associations, mining associations, and schools are concerned that health risks of some of the minerals might be considered as equivalent to the risks posed by commercial types of asbestos. Regulatory agencies are faced with difficult decisions in the absence of sound scientific data to assess the risk of low-level exposure to diverse types of mineral fibers.

While recognizing that asbestos is an established human carcinogen, further animal studies are needed to better understand toxic and carcinogenic risk. The inherent limitations in deriving risk estimates from human studies due to incomplete exposure information leads to uncertainty in the dose-response relationship and ultimately in the potency and risk for a particular mineral fiber exposure. Evidence from human studies for cancers at sites outside the respiratory tract and non-malignant diseases such as autoimmune disorders is suggestive but these studies do not allow definitive causal relationships to be established for these diseases at this time. Animal studies will help elucidate the potential hazard as well as dose-response relationships and relative potency for specific mineral fibers for which no health effects data exist. The U.S. EPA National Health and Environmental Effects Laboratory (NHEERL) and the National Institute for Occupational Safety and Health (NIOSH) are both developing research programs related to mineral fibers. The proposed NTP research program on mineral fibers is intended to complement these other federal research efforts. There is an established interagency asbestos working group that is serving as a forum for coordinating research among the different federal agencies. This working group will continue to provide input to the NTP in order to ensure that the proposed research program will meet public health and regulatory agency data needs. Additional efforts to take advantage of the considerable expertise external to the NTP in asbestos and fiber toxicology will be important in designing specific studies. Developing collaborative research projects with academic investigators ancillary to the NTP toxicology studies is another option that will be considered for increasing the scope and value of this research program.

### **Key Issues**

The overarching issue raised by this nomination is the relative contribution of mineralogy, morphology and dimension to the toxicity of diverse natural mineral fibers. Each of these attributes influences fiber durability, deposition, and clearance, and thus persistence in the body. Besides biopersistence, inherent bioreactivity due to mineral fiber composition will influence toxicity. To tease out relationships among physico-chemical attributes and toxicity, thorough characterization of mineralogy, morphology and dimension at all phases of study will be critical. There is also an incomplete understanding of fiber deposition and clearance in humans and animal models following inhalation exposure and whether an internal dose metric such as cumulative lung burden may be a more appropriate metric for evaluating risk. Few studies have evaluated translocation of fibers outside the respiratory tract, key to understanding pathogenesis of extrapulmonary diseases. The relative toxicity of non-asbestiform particles meeting the regulatory fiber definition can only be directly addressed by testing relatively pure samples with the same mineral composition and particle dimension. This issue can be indirectly addressed, however, by testing multiple samples with a range of morphologies, dimensions and composition.

As evident from the previous discussion, addressing the key issues for this nomination relies on the identification, collection, and processing of suitable mineral fiber test materials. Site-specific mineral sample mixtures such as the Libby amphibole and the El Dorado material as well as mineralogically pure samples with mixed morphology and dimension will be sought. Specific considerations include:

- Methods of processing of mineral samples to generate test materials; e.g. whether samples should be enriched for long fibers or rat respirable fibers as specified in internationally

accepted fiber test guidelines. Accessory mineral particles such as calcite or quartz are likely to be present in these samples and may contribute to the adverse human health effects of NOA exposure.

- Value in attempting to obtain non-asbestiform amphibole mineral samples containing elongated particles with fiber-like dimensions (e.g. tremolite acicular and prismatic crystals, cleavage fragments).
- Whether test materials could be partially purified (size separated) without loss of bioactive structures, i.e. by altering elemental composition, surface chemistry, or dimensional distribution.

### **Proposed Approach**

The goal of this research program is to provide robust toxicological characterizations (dose-response for cancer and non-cancer endpoints) of several representative natural mineral fibers by conducting long-term inhalation studies in a rodent model. Hypotheses to be addressed include:

- Cumulative dose, as measured by lung fiber burden, is the most appropriate dose metric for predicting carcinogenic and non-carcinogenic effects of natural mineral fibers.
- The cumulative dose-response and relative potency differs among representative natural mineral fibers.
- The degree of *in vivo* biopersistence of natural mineral fibers is a function of dimension, morphology, and mineralogy, and is predictive of toxic and carcinogenic potency.
- Particles with similar dimension and chemical composition (mineralogy), but of differing morphology, have equivalent toxic and carcinogenic potencies.
- Particles with similar dimension and morphology, but of differing chemical composition (mineralogy), have equivalent toxic and carcinogenic potencies.

The specific aims of the proposed research program are as follows:

Identify sources of representative natural mineral fibers. The USGS Denver Microbeam Facility, through an interagency agreement with the U.S. EPA, will be collecting material from the Libby mine (“Libby amphibole composite”) in late summer 2007. Previous sampling and analysis by the USGS has demonstrated the feasibility of acquiring sufficient material for toxicology studies that is representative of the range of amphiboles present in soil and air samples from the Libby area. Sufficient material will be generated for the proposed EPA/NHEERL and NTP studies. The NTP, in consultation with the USGS, ATSDR and EPA, will identify 3-4 other natural mineral fibers and sources from which to prepare suitable test materials for long-term toxicology studies. Possible sources include winchite from the Libby area, ferroactinolite or grunerite asbestos from Minnesota, erionite from Oregon or South Dakota, and surrogate tremolite and actinolite samples that are representative of the amphiboles found in El Dorado, California. The goal in collecting these different materials will be to both address site-specific human health concerns and acquire a set of mineral samples that represent a broad range of mineralogy, morphology and dimension. As with the Libby material, it will be important to have comparable characterization data for materials collected for toxicity studies and site-specific environmental sampling. Commercially available asbestos “standard” materials that have been adequately characterized previously (e.g. amosite or tremolite asbestos) will also be included as test materials for comparison to toxicity studies in the literature.

Conduct physical and chemical characterizations of test materials (mineralogy, morphology).

The USGS will characterize the Libby amphibole composite and advise the NTP on appropriate analytical methods for characterizing other mineral samples. Relevant characteristics include distribution of particle morphology, length and diameter, and mineral composition in a given sample by microscopic (PCM, SEM, TEM), X-ray diffraction, and electron probe microanalysis methods. Methods for assessing surface chemistry and reactivity will also be explored.

Obtain fiber durability and *in vitro* toxicity data. Studies of *in vitro* dissolution of mineral fibers in simulated biological fluids or other media are used to assess fiber durability and can facilitate interpretation of *in vivo* biopersistence studies. For example, durable particles may initially deposit in the lung but be effectively cleared, while non-durable particles will not persist even if initially deposited in the lung. The USGS and EPA/NHEERL have conducted or are planning to conduct dissolution and *in vitro* cellular toxicity studies for the Libby amphibole and a range of commercially available asbestos standards. It is anticipated that some of these data will be available for use in prioritizing and designing toxicity studies. Amphibole particles present in these samples are anticipated to be durable, thereby limiting the predictive utility of acellular dissolution assays. However, consideration will be given to conducting a limited number of dissolution studies for other mineral fibers selected for toxicity studies. While acknowledging the limited value of *in vitro* toxicity assays for predicting chronic toxicity of fibers, such studies could provide insight into mechanisms of genotoxic and inflammatory responses. *In vitro* genotoxicity assays are proposed as part of this program. Conducting additional *in vitro* toxicity studies through collaboration with EPA/NHEERL investigators will also be explored.

Conduct short-term *in vivo* biopersistence studies. Biopersistence will be assessed by evaluating lung and pleural fiber burden following 5 or 14 day inhalation exposures and a 1-90 day observation period. These studies will be used to estimate lung clearance half-life and for setting doses for subchronic studies. The degree of biopersistence has been used as an indicator of potential carcinogenic activity of mineral fibers. Quantification of all fiber types and sizes in these studies will yield data on the relative biopersistence of different elongated mineral particles within a given samples and between different test materials. This will allow for further validation of predictive toxicity models for evaluating relative potency of different type and size fibers.

Conduct subchronic and chronic toxicology studies. Subchronic and chronic nose-only inhalation studies will be conducted in Wistar Han rats for the Libby amphibole composite, a commercial asbestos material, and 1-2 other selected mineral fibers. Selection of test materials will be based on the mineral characterization and biopersistence studies, with attention to specific physico-chemical attributes that will allow key study hypotheses to be addressed. Subchronic and chronic studies will include special assessments of genotoxicity, pulmonary function and toxicity (bronchioalveolar lavage, cytokine measurement, markers of cytotoxicity, inflammation, and cell proliferation), and full characterization of mineral particles in lung, pleura and selected extrapulmonary tissues. Chronic dose-response studies will be performed with a minimum of 90 days continuous exposure duration. Because of public health concern for children's exposures at NOA sites, studies where exposures begin as early in life as possible (e.g. 3 weeks of age) will be considered. The duration of exposure will be a critical element in testing hypotheses related to cumulative dose and optimal dose metrics for evaluating risk. Thus, other exposure regimens will be considered for the chronic studies, and include treatment groups with variable

concentration (C) and exposure duration (T), and with a cumulative administered dose (C x T) in the same range as the C x T for continuous exposure treatment groups. Extending the duration of observation period beyond 2 years (e.g. until natural death or 80% mortality in any dose group) may increase the incidence of lung tumors and mesothelioma, thereby enhancing the sensitivity of these studies for evaluating relative potency for these endpoints among different test materials.

### **Significance and Expected Outcome**

There remains considerable uncertainty in the dose-response for adverse health effects of asbestos and related mineral fibers, and by extension whether current environmental and occupational exposure standards are adequately protective of human health. There are likewise many gaps in current knowledge related to the physical and chemical determinants of mineral fiber toxicity and the mechanisms of fiber-induced adverse effects. A complete or even adequate understanding of the influence of fiber durability, biodisposition and inherent bioreactivity on toxic outcomes is beyond the scope of any one set of studies and will only be gained through long term and continued investment in fiber research. This NTP research program is designed to address some key outstanding issues regarding hazards of natural mineral fibers and contribute to advancing the field of fiber toxicology.

Regulatory efforts are in progress to revise health risk guidance values to account for mineral fiber type and size. The result of the proposed toxicology and carcinogenesis studies will be published as peer-reviewed NTP Technical Reports and data from the NTP studies can be directly used in human health risk assessment. A few examples of direct public health impact follow. The U.S. EPA, through its IRIS program, is reassessing asbestos risk and intends to develop new RfC and carcinogenic unit risk values for asbestos fibers. These risk values are used by other EPA Offices and Regions when conducting site-specific risk assessments and determining the need for cleanup actions. The ATSDR develops Minimal Risk Values (MRVs) for hazardous substances which are used when providing guidance to communities as part of ATSDR public health consultations. The NIOSH Asbestos and Other Mineral Fibers Roadmap identifies a number of research areas that will “serve as the basis for evidence-based public health policies for asbestos and other mineral fibers” (NIOSH, 2007). Several of the research areas described in the NIOSH Roadmap will be addressed by the proposed NTP research program. Lastly, there are many types of natural mineral fibers and synthetic fibers, including those generated through nanotechnology, for which adequate toxicological data do not exist. A better understanding of the determinants of fiber toxicity developed through this research program will instill greater confidence in the use of short-term toxicological methods for assessing the hazards of these fibers.

### **References and Supporting Materials**

Agency for Toxic Substances and Disease Registry (2001). Toxicological Profile for Asbestos. Available at <http://www.atsdr.cdc.gov/toxprofiles/tp61.html>.

ATSDR (2003a). Public Health Assessment. Libby Asbestos Site, Libby, Lincoln County, Montana. EPA Facility ID: MT0009083840. Available at [http://www.atsdr.cdc.gov/HAC/pha/libby3/lby\\_toc.html](http://www.atsdr.cdc.gov/HAC/pha/libby3/lby_toc.html).

ATSDR (2003b). Report on the Expert Panel on Health Effects of Asbestosis and Synthetic Vitreous Fibers: The Influence of Fiber Length. Available at <http://www.atsdr.cdc.gov/HAC/asbestospanel/>.

ATSDR (2006). Health Consultation. Asbestos Exposures At Oak Ridge High School, 1120 Harvard Way, El Dorado Hills, California. EPA Facility ID: CAN000906055. Available at <http://www.atsdr.cdc.gov/sites/eldoradohills/index.html>

ATSDR (2007). Locations of Naturally Occurring Asbestos. <http://www.atsdr.cdc.gov/NOA/AsbestosFound.html>.

Bernstein, D., V. Castranova, K. Donaldson, B. Fubini, J. Hadley, T. Hesterberg, A. Kane, D. Lai, E.E. McConnell, H. Muhle, G. Oberdorster, S. Olin, and D.B. Warheit (2005). Testing of Fibrous Particles: Short-Term Assays and Strategies: Report of an ILSI Risk Science Institute Working Group. *Inhal. Toxicol.* 17:497-537. [PMID: 16040559](#).

European Chemicals Bureau (1999). Methods for the Determination of Hazardous Properties for Human Health of Man Made Mineral Fibres (MMMMF), European Commission Joint Research Centre Report EUR 18748 EN (1999), eds. DM Bernstein and JM Riego Sintes. Available at <http://ecb.jrc.it/DOCUMENTS/Testing-Methods/mmmfweb.pdf>

Meeker GP, Bern AM, Brownfield IK, Lowers HA, Sutley SJ, Hoefen TM, and Vance, JS. (2003). The composition and morphology of amphiboles from the Rainy Creek complex, near Libby, Montana. *Am Mineralogist* 88:1955–1969. Available at [http://www.minsocam.org/MSA/AmMin/TOC/Articles\\_Free/2003/Meeker\\_p1955-1969\\_03.pdf](http://www.minsocam.org/MSA/AmMin/TOC/Articles_Free/2003/Meeker_p1955-1969_03.pdf).

Institute of Medicine (2006). Asbestos: Selected Cancers. Board on Population Health and Public Health Practice, Committee on Asbestos: Selected Health Effects. The National Academies Press, Washington, D.C. Available at <http://www.nap.edu/catalog/11665.html>.

National Institute for Occupational Safety and Health (2007). Asbestos and Other Mineral Fibers: A Roadmap for Scientific Research. Available at <http://www.cdc.gov/niosh/review/public/099/pdfs/NIOSHAsbestosRoadmap.pdf>.

Sullivan, P.A. (2007). Vermiculite, respiratory disease and asbestos exposure in Libby, Montana: Update of a cohort mortality study. *Environ. Health Perspect.* 115:579-585. [PMID: 17450227](#). Available at <http://www.ehponline.org/members/2007/9481/9481.html>.

U.S. Environmental Protection Agency (2001). FIFRA Scientific Advisory Panel Meeting, September 26, 2000. Test Guidelines for Chronic Inhalation Toxicity and Carcinogenicity of Fibrous Particles. SAP Report No. 2001-01. Available at [http://www.epa.gov/scipoly/sap/meetings/2000/september/final\\_fibers.pdf](http://www.epa.gov/scipoly/sap/meetings/2000/september/final_fibers.pdf).

U.S. EPA (2005). El Dorado Hills, Naturally Occurring Asbestos Multimedia Exposure Assessment. El Dorado Hills, California. Preliminary Assessment and Site Inspection Report Interim Final. Available at <http://www.epa.gov/region09/toxic/noa/eldorado/index.html>.

U.S. EPA Office of the Inspector General (2006). EPA Needs to Plan and Complete a Toxicity Assessment for the Libby Asbestos Cleanup. Report No. 2007-P-00002. December 5, 2006. Available at <http://www.epa.gov/oig/reports/2007/20061205-2007-P-00002.pdf>.

U.S. EPA Office of Research and Development (2006). NTP Nomination Letter from George Gray dated December 11, 2006. <http://ntp.niehs.nih.gov/index.cfm?objectid=00817588-F1F6-975E-7D64F49FA6ED1D79>.

U.S. Geological Survey (2006). Mineralogy and Morphology of Amphiboles Observed in Soils and Rocks in El Dorado Hills, California. Open-File Report 2006-1362. Available at <http://pubs.usgs.gov/of/2006/1362/>.

September 2007